

# Effect of stimulus configuration on crowding in strabismic amblyopia

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**Foveal vision in strabismic amblyopia can show increased levels of crowding, akin to typical peripheral vision. Target-flanker similarity and visual-acuity test configuration may cause the magnitude of crowding to vary in strabismic amblyopia. We used custom-designed visual acuity tests to investigate crowding in observers with strabismic amblyopia. LogMAR was measured monocularly in both eyes of 11 adults with strabismic or mixed strabismic/anisometropic amblyopia using custom-designed letter tests. The tests used single-letter and linear formats with either bar or letter flankers to introduce crowding. Tests were presented monocularly on a high-resolution display at a test distance of 4 m, using standardized instructions. For each condition, five letters of each size were shown; testing continued until three letters of a given size were named incorrectly. Uncrowded logMAR was subtracted from logMAR in each of the crowded tests to highlight the crowding effect. Repeated-measures ANOVA showed that letter flankers and linear presentation individually resulted in poorer performance in the amblyopic eyes (respectively, mean normalized logMAR = 0.29,  $SE = 0.07$ , mean normalized logMAR = 0.27,  $SE = 0.07$ ;  $p < 0.05$ ) and together had an additive effect (mean = 0.42,  $SE = 0.09$ ,  $p < 0.001$ ). There was no difference across the tests in the fellow eyes ( $p > 0.05$ ). Both linear presentation and letter rather than bar flankers increase crowding in the amblyopic eyes of people with strabismic amblyopia. These results suggest the influence of more than one mechanism contributing to crowding in linear visual-acuity charts with letter flankers.**

changes (Barrett, Bradley, & McGraw, 2004; McKee et al., 1992). It is characterized by deficits in visual acuity and contrast sensitivity (Asper, Crewther, & Crewther, 2000), crowding (Bonneh, Sagi, & Polat, 2004; Levi & Klein, 1985; Whitney & Levi, 2011), fixation and ocular motility (Birch, 2013; Carpineto et al., 2006; Ciuffreda, Levi, & Selenow, 1991; González, Wong, Niechwiej-Szwedo, Tarita-Nistor, & Steinbach, 2012; Schor, 1975; Schor & Hallmark, 1978), temporal processing (Bonneh et al., 2007; Tkacz-Domb & Yeshurun, 2017), global motion perception (Meier, Sum, & Giaschi, 2016), spatial localization (Hess & Holliday, 1992; Levi, Klein, & Yap, 1987), spatial interactions (Polat, Sagi, & Norcia, 1997), and contour integration (Kovács, Polat, Pennefather, Chandna, & Norcia, 2000).

Crowding is a reduction in the ability to recognize objects in the midst of clutter and is present in everyday vision (Flom, 1991; Levi, 2008). A number of factors can contribute to the overall crowding effect, including the proximity of the flanking elements to the target, similarity of the target and flankers, and overall target-flanker configuration (Dakin, Cass, Greenwood, & Bex, 2010). Here we refer to contour interaction as the specific reduction in visual acuity caused by the proximity of simple flanking elements (Flom, Weymouth, & Kahneman, 1963), which is governed by the spacing of the target and flankers. This paper explores the effect of two other aspects of visual-acuity chart design on crowding in participants with strabismic amblyopia: target-flanker similarity (letter or bar) and configuration of letters (single or linear).

The use of crowded visual acuity tests has long been advocated to avoid the potential overestimation of acuity arising from the use of single, unflanked optotypes (Flom, 1991; Hilton & Stanley, 1972; Youngson, 1975), but the overall level of crowding

## Introduction

Amblyopia is a developmental syndrome, whereby neuroplasticity at birth drives structural and functional

Citation: Norgett, Y., & Siderov, J. (2017). Effect of stimulus configuration on crowding in strabismic amblyopia. *Journal of Vision*, 17(13):5, 1–14, doi:10.1167/17.13.5.

doi: 10.1167/17.13.5

Received March 20, 2017; published November 06, 2017

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varies according to the configuration of the test components (Formankiewicz & Waugh, 2013; Norgett and Siderov, 2011). Crowding is achieved in visual acuity tests by placing either bars or optotypes around a single target optotype or by using rows of optotypes—either in a traditional chart format or presented one row at a time—with a surround box providing a contour around the optotypes. Crowded acuity tests of different designs have been judged to be equivalent to each other because they give a similar result, but the risk is that they may not give a similar result in individuals with abnormal crowding, such as young children or those with strabismic amblyopia (Stager, Everett, & Birch, 1990).

Crowded acuity has a longer timescale of maturation in children than uncrowded acuity (Doron, Spierer, & Polat, 2015; Drover et al., 2008; Langaas, 2011; Morad, Werker, & Nemet, 1999; Norgett & Siderov, 2014; Pan et al., 2009). During typical maturation of the visual system, a decrease in crowding is accompanied by an improvement in higher level functions such as contour integration and lateral interactions (Doron et al., 2015; Kovács, Kozma, Fehér, & Benedek, 1999). It follows that visual acuity measured using more complex targets—such as full letter charts, which require higher level processing—may not be expected to show maturity until later than is the case for simple targets and may therefore be more affected in amblyopia.

One of the questions addressed in this study is whether similarity of targets and flankers affects crowding in foveal or extrafoveal vision in strabismic amblyopia. Our previous work has shown that use of letter rather than bar flankers increases crowding in foveal vision in young children, but not in adults with typical vision (Norgett & Siderov, 2014). There is evidence that target–flanker similarity increases crowding in the retinal periphery for lower level features such as contrast polarity, shape, depth, contrast, and color (Astle, McGovern, & McGraw, 2014; Chung, Levi, & Legge, 2001; Kennedy & Whitaker, 2010; Kooi, Toet, Tripathy, & Levi, 1994; Nazir, 1992), for higher level features such as faces (Farzin, Rivera, & Whitney, 2009) and global configuration (Livne & Sagi, 2011), and for letter targets (Bernard & Chung, 2011; Freeman, Chakravarthi, & Pelli, 2012). In strabismic amblyopia, foveal crowding has been found to be greater in extent than in typical eyes and has been likened to crowding in the periphery in typical vision (Flom, 1991; Levi & Klein, 1985). Some authors have reported that when scaled to individual resolution threshold, crowding is similar for amblyopic and typical eyes (Flom et al., 1963; Simmers, Gray, McGraw, & Winn, 1999), but others have reported that in amblyopic vision, the extent of crowding is greater than even the reduced acuity would

predict (Hariharan, Levi, & Klein, 2005; Hess, Dakin, Tewfik, & Brown, 2001; Levi, Hariharan, & Klein, 2002). Studies in amblyopia performed with children (Greenwood et al., 2012) and adults (Bonneh et al., 2004) have reported both results; excessive crowding was found in individuals with strabismic and mixed strabismic/anisometropic amblyopia but not with pure anisometropic amblyopia.

The task of reading across a line of letters in a visual acuity test rather than naming a single crowded letter places demands which may disproportionately disadvantage a reader with strabismic amblyopia over one with typical vision. Abnormal gaze control may reduce acuity in line charts or reading long strings of letters, where accurate fixation is required (Bedell, Siderov, Formankiewicz, Waugh, & Aydin, 2015; Giaschi, Regan, Kraft, & Kothe, 1993; Regan, Giaschi, Kraft, & Kothe, 1992). Kanonidou, Proudlock, and Gottlob (2010) measured reading speed and tracked eye movements in observers with strabismic amblyopia and compared the results to those from observers without amblyopia. They found that those with strabismic amblyopia made more saccades per line than those in the control group. In contrast to the conclusions of Levi, Song, and Pelli (2007), that reduced reading speeds in those with amblyopia can be explained fully by crowding effects (letter spacing), Kanonidou et al. concluded that those slower reading speeds could not be accounted for solely by spacing. The study by Levi et al. used rapid serial visual presentation to eliminate the effect of eye movements, so it could be that oculomotor deficits added a hindrance to amblyopic reading in addition to the spatial deficit introduced by contour interaction.

An alternative or additional factor which could disadvantage a person with amblyopia in reading a line test is positional uncertainty, which could cause misnaming of letters. Positional uncertainty can be thought of as an imprecision in the focus of attention, which increases with increasing eccentricity in typical vision (Strasburger, 2005). Positional uncertainty has been shown to be greater in strabismic amblyopia than in typical vision (Hess, McIlhagga, & Field, 1997; Levi et al., 1987), as a result of neural undersampling (Levi & Klein, 1986) or disruption of cortical topography (Hess, 1982; Kiorpes & McKee, 1999).

In the present study, visual acuity was measured in both eyes of adults with strabismic and mixed strabismic/anisometropic amblyopia using custom-designed tests to investigate the relative contributions of target–flanker similarity and linear versus single-letter presentation to the crowding effect. Analysis of letter-identification errors enabled comparison between fixation behavior in amblyopic and fellow eyes.

| Initials | Age/<br>gender | Eye (type)           | Surgery/<br>patching | Alignment   | Stereopsis<br>(in.) | Refractive error                           | logMAR        |
|----------|----------------|----------------------|----------------------|---|---------------------|--|---------------|
| KW       | 56/F           | R (strabismus)       | No/yes               | Microtropia   | 600                 | R +2.50<br>L +2.50/−0.50 × 90              | 0.62<br>−0.14 |
| FD       | 39/F           | R (strabismus)       | Yes/yes              | Microtropia   | 200                 | R −1.00/−1.00 × 180<br>L −0.50/−0.75 × 140 | 0.44<br>−0.08 |
| MP       | 40/M           | L (strabismus)       | No/yes               | 8 <sup>^</sup> esotropia  |                     | R +0.50/−0.50 × 170<br>L plano             | −0.20<br>0.80 |
| BS       | 75/F           | R (strabismus)       | No/yes               | 12 <sup>^</sup> esotropia   |                     | R +2.50/−0.25 × 110<br>L +2.25/−0.25 × 70  | 0.36<br>0.10  |
| JP       | 62/M           | L (strabismus)       | No/yes               | 3 <sup>^</sup> esotropia<br>2 <sup>^</sup> hypotropia                               |                     | R +2.00/−1.25 × 170<br>L +1.75             | 0.00<br>0.56  |
| JB       | 61/F           | R (strabismus/mixed) | Yes/yes              | 5 <sup>^</sup> esotropia  |                     | R +3.75/−0.50 × 80<br>L +1.00/−1.25 × 95   | 0.60<br>0.00  |
| PD       | 49/F           | L (strabismus/mixed) | No/no                | 4 <sup>^</sup> esotropia  |                     | R −2.50/−1.00 × 160<br>L +0.75/−0.50 × 10  | −0.08<br>0.44 |
| PG       | 69/M           | L (strabismus/mixed) | Yes/yes              | 6 <sup>^</sup> esotropia  |                     | R plano/−1.25 × 80<br>L +2.50/−1.25 × 10   | 0.02<br>0.66  |
| LM       | 23/F           | R (strabismus/mixed) | Yes/yes              | 40 <sup>^</sup> accommodative<br>esotropia  |                     | R +7.00/−2.00 × 40<br>L +6.50/−0.50 × 140  | 0.10<br>−0.10 |
| TW       | 43/M           | R (strabismus/mixed) | Yes/yes              | Microtropia with Rx<br>4 <sup>^</sup> R hypotropia and 10 <sup>^</sup><br>esotropia |                     | R +1.50<br>L plano                         | 0.92<br>0.02  |
| MOL      | 37/F           | L (strabismus/mixed) | No/yes               | 24 <sup>^</sup> accommodative<br>esotropia; microtropia<br>with Rx                  |                     | R +5.00/−3.50 × 157<br>L +6.50/−5.00 × 20  | 0.20<br>0.78  |

Table 1. Clinical characteristics of the participants. *Notes:* Microtropia: presumed microtropia—no movement detected on cover test; <sup>^</sup> prism diopters.

## Methods

### Participants

Five adult participants with strabismic amblyopia and six with mixed strabismic/anisometropic amblyopia were recruited from the local community. The number of participants was sufficient to obtain a power of 80% at the 5% level (one tailed) for an effect size of 0.15 logMAR. For the purposes of this study, amblyopia was defined as at least a two-line difference in visual acuity between the eyes of participants, in the presence of strabismus or anisometropia (or both) and in the absence of structural abnormality of the eye or visual pathway. All participants underwent a detailed optometric assessment prior to experimental testing, including fundus examination, subjective refraction, logMAR acuity (Thompson logMAR chart, Thomson Software Solutions, Hatfield, Hertfordshire, UK), stereopsis using the Lang II Stereotest (Lang-Stereotest, Küsnacht, Switzerland), and cover test for distance and near fixation. Any observed heterophoria or heterotropia was measured using a prism cover test. History of previous treatment, if any, was also recorded. Clinical details of the participants are given

in Table 1. Written, informed consent was obtained from participants after all the procedures were explained to them. Ethical approval for the study was obtained from the university research ethics Panel, and the study followed the tenets of the Declaration of Helsinki.

### Tests

A series of letter tests was produced, comprising single letters and lines of letters with letter and bar flankers (Table 2). An unflanked condition was also included. The test optotypes comprised the set of 10 Sloan letters (C, D, H, K, N, O, R, S, V, and Z), constructed in a 5 × 5 format, with the height and the width of each letter 5 times the stroke width. Edge-to-edge separation of letters and flankers for all conditions was 0.5 letter width, in order to produce crowding (Norgett & Siderov, 2014). The length of the bar flankers was fixed at 0.6 times letter height (i.e., three stroke widths), in order to maintain the same average contour interaction across different flankers. The distance of flanking features to a target influences the possibility of integration of the flankers with target, with flankers close to the target being more heavily



| Test           | Sloan-letter target       | Flanker type         | Example display                                |
|----------------|---------------------------|----------------------|--|
| S <sub>0</sub> | Single                    | None                 | H  |
| SB             | Single                    | Bars                 | ┌─┐<br>└─┘                                     |
| LB             | Linear<br>(five letters)  | Bars                 | ┌─┐┌─┐┌─┐┌─┐┌─┐<br>└─┘└─┘└─┘└─┘└─┘             |
| LB7            | Linear<br>(seven letters) | Bars                 | ┌─┐┌─┐┌─┐┌─┐┌─┐┌─┐┌─┐<br>└─┘└─┘└─┘└─┘└─┘└─┘└─┘ |
| SC             | Single                    | Non-Sloan characters | M<br>AKB<br>J                                  |
| SCsl           | Single                    | Sloan characters     | K<br>DHO<br>Z                                  |
| LC             | Linear                    | Non-Sloan characters | F Q U Y A<br>R O H D N S K<br>B W J M T        |

Table 2. Tests used in the study, with an example presentation of each. *Notes:* Letters were presented in single (S) or linear (L) format with bar (B) or character (C) flankers. The edge-to-edge letter-flanker separation was 0.5 letter width in each of the crowded tests.

weighted (Dakin et al., 2010; Takahashi, 1968). Hence, to ensure consistency of the size of the nearest edge between letter and bar flankers, we considered each of the four sides of the virtual box surrounding each letter and identified where there was contact with the edge of the letter. For example, consider the letter *H* in Sloan format: There are five stroke widths' contact with the edge of the virtual box on the right and left sides and two on the top and bottom, giving a mean of 3.5 stroke widths' contact over the four sides of the letter. For circular letters, we took an approximation because of curvature. The stroke width of the edges of all the letters was averaged to derive the value of three stroke widths, or 0.6 times the letter height.

The following between-test comparisons were made: (i) S<sub>0</sub> with SB to determine the magnitude of contour interaction; (ii) SB with SC, and LB and LB7 with LC to determine the effect of letter rather than bar flankers; and (iii) SB with LB and LB7, and SC with LC to determine the effect of single-letter versus linear presentation.

In comparisons ii and iii, contour interaction was kept constant by maintaining a fixed edge-to-edge target-flanker distance. In LB and LB7, the bar flanker between letters was retained so that the nearest contour to each letter would always be a bar at 0.5 letter width's separation from the target letter, as in SB. In test SC the task was to read the central letter only, and in test

LC the task was to read the letters on the middle line. However, because the end letters in LC are flanking letters, only the middle five letters were scored. Test LB7 was created to enable comparison between flanker types in linear tests with seven letters. Tests SC and LC used 14 non-Sloan letter flankers, created in the same format as Sloan letters. The letters *I* and *G* were not used, because the *I* does not contact the outside of the virtual box on either side and the appearance of the *G* in this format was judged to be unusual. In addition, a version of the single-letter chart with letter flankers, SC, was created using Sloan flankers and named SCsl. This was to allow further error analyses, because when non-Sloan letter flankers were used, the procedure did not allow for non-Sloan letter responses.

Tests were displayed, with black letters on a white background, on an Apple iMac 21.5-in. screen (Apple Inc., Cupertino, CA) with a resolution of 1,920 × 1,080 pixels at 102.46 pixels/in., so 1 pixel subtended 0.2 arcmin at a test distance of 4 m. Background luminance of the display was 266 cd/m<sup>2</sup>, resulting in a letter Weber contrast of ~92%. The acuity range of the tests was 0.6 logMAR to ~0.4 logMAR in steps of 0.05 logMAR. For each acuity level, five letters were scored in each test. In the single-letter presentations, five different letters of the same size were shown consecutively.

## Procedure

Testing was carried out in a room with lighting adequate for visual acuity testing (National Academy of Sciences National Research Council Committee on Vision, 1980), approximately 100 lux. Following refraction and screening tests, the experimental tests were viewed, separately, by each eye of eligible participants wearing their best corrective lenses. For each participant, the nonamblyopic eye was tested first. The eye not being tested was occluded. Participants sat 4 m from the screen and held a card showing the 10 Sloan letters. A testing distance of 4 m was chosen to maximize largest letter size within the confines of the screen size and test room. The eight experimental tests were presented in a random order across individuals, and viewing time was unlimited. Testing began using a letter size 0.1 logMAR larger than the acuity measured following refraction. Smaller letter sizes were presented in steps of 0.05 logMAR until the termination point was reached, when three or more letters of one size were named incorrectly. If any letters at the starting level were named incorrectly, the next largest size was presented until a size was found where all five responses were correct. Where the largest letters (0.6 logMAR) were not all read correctly at 4 m, the viewing distance was decreased to 2 m, with the participant's refractive correction and

logMAR score adjusted appropriately. For the single-letter tests with letter flankers (SC and SCsl), participants were asked to read the middle letter only (Table 2). For the line test with letter flankers (LC), participants were asked to read all the letters on the middle row, but only the central five letters were scored. In the line test with seven letters and bar flankers (LB7), only the central five letters were scored. If a participant was not sure of a letter, they were encouraged once to guess—or in the tests with letter flankers (SC and LC), if they named a non-Sloan letter they were directed to retry from the Sloan-letter set. Pointing at the letters by the examiner was not used under any test condition.

All responses were recorded on a spreadsheet by the examiner, and letter-by-letter scoring was used. For the line tests, if a participant read an incorrect number of letters in a line without indicating that they were leaving one out, the responses were recorded in the order and position they were read. Results were normalized by subtracting the unflanked logMAR ( $S_0$ ) from the logMAR derived from each of the crowded tests, to allow comparison of the crowding effect between the tests.

## Data analysis

Data were analyzed using paired  $t$  tests and repeated-measures ANOVA with a Greenhouse–Geisser correction for violation of sphericity applied (Keppel, 1982). Post hoc analyses with Tukey's honest significant difference correction were performed as required (Statistica StatSoft, Tulsa, OK). Letter-naming errors were also analyzed in the three line tests (LB, LB7, LC) and in the single-letter test with letter flankers (SCsl) to investigate any difference in pattern between tests and amblyopic or fellow eyes. Errors were defined as either adjacent, if the response letter was adjacent to the target letter (either left or right, top or bottom), or random, if any other letter was named. In the line tests with bar flankers (LB and LB7), errors pertaining to just the central three or five letters (respectively) were analyzed, because the end letters had only one possible adjacent option. In the line test with letter flankers (LC), errors pertaining to the central five letters were analyzed. Four analyses were carried out. The first examined whether the adjacent errors in the line tests (LB, LB7, LC) were anything other than random; the second examined the frequency of right and left adjacent errors in line tests LB and LC; the third examined whether the adjacent errors in the single-letter test with Sloan-letter flankers (SCsl) were anything other than random; and the fourth looked for a difference in the frequency of adjacent and random errors between amblyopic and fellow eyes in test SCsl.

Chi-square tests were performed to assess statistical significance at a level of  $p < 0.05$ .

## Results

### Individual participants

Figure 1 shows the logMAR for each individual, normalized to the unflanked logMAR, for each of the crowded tests. Visual acuity in the amblyopic eye, as found from the initial screening, is displayed in the top right-hand side of each panel. The panels are arranged in order of increasing depth of amblyopia. There was a general trend toward more crowding in participants with deeper amblyopia, consistent with the findings of Bonneh et al. (2004).

As expected, the mean logMAR for the sample was better than 0.00 (6/6) in the nonamblyopic (fellow) eyes in all the tests. Before we normalized the results, paired  $t$  tests were performed to look for a difference between the uncrowded condition ( $S_0$ ) and the single-letter condition with bar flankers (SB). There was a significant difference between these two tests for both amblyopic and fellow eyes ( $p < 0.05$ ), showing an effect of contour interaction in each. When logMAR was normalized to the unflanked condition, there was no significant difference between amblyopic and fellow eyes using test SB ( $p = 0.43$ ), showing, on average, no additional contour interaction in the amblyopic eyes relative to the fellow eyes in this condition.

Figure 2 and Table 3 show mean, normalized logMAR across the test conditions for amblyopic and fellow eyes. A one-way repeated-measures ANOVA for the amblyopic eyes yielded a significant main effect of test,  $F(2.11, 21.15) = 12.47$ ,  $p < 0.001$ . A post hoc analysis showed mean, normalized logMAR in the amblyopic eyes in the single-letter condition with bar flankers (SB) to be different from all the other tests ( $p < 0.05$ ). There was no difference in mean normalized logMAR in the nonamblyopic eyes across any of the tests,  $F(3.39, 33.94) = 0.75$ ,  $p = 0.59$ .

### Effect of letter versus bar flankers (target–flanker similarity)

In the amblyopic eyes, there was more crowding in the tests with letter rather than bar flankers. For the single-letter tests, mean logMAR was significantly poorer in the test with letter flankers (SC) than the one with bar flankers (SB;  $p < 0.05$ ). In the line tests, there was also more crowding with letter than bar flankers; mean, normalized logMAR was significantly worse in LC than LB ( $p < 0.05$ ).

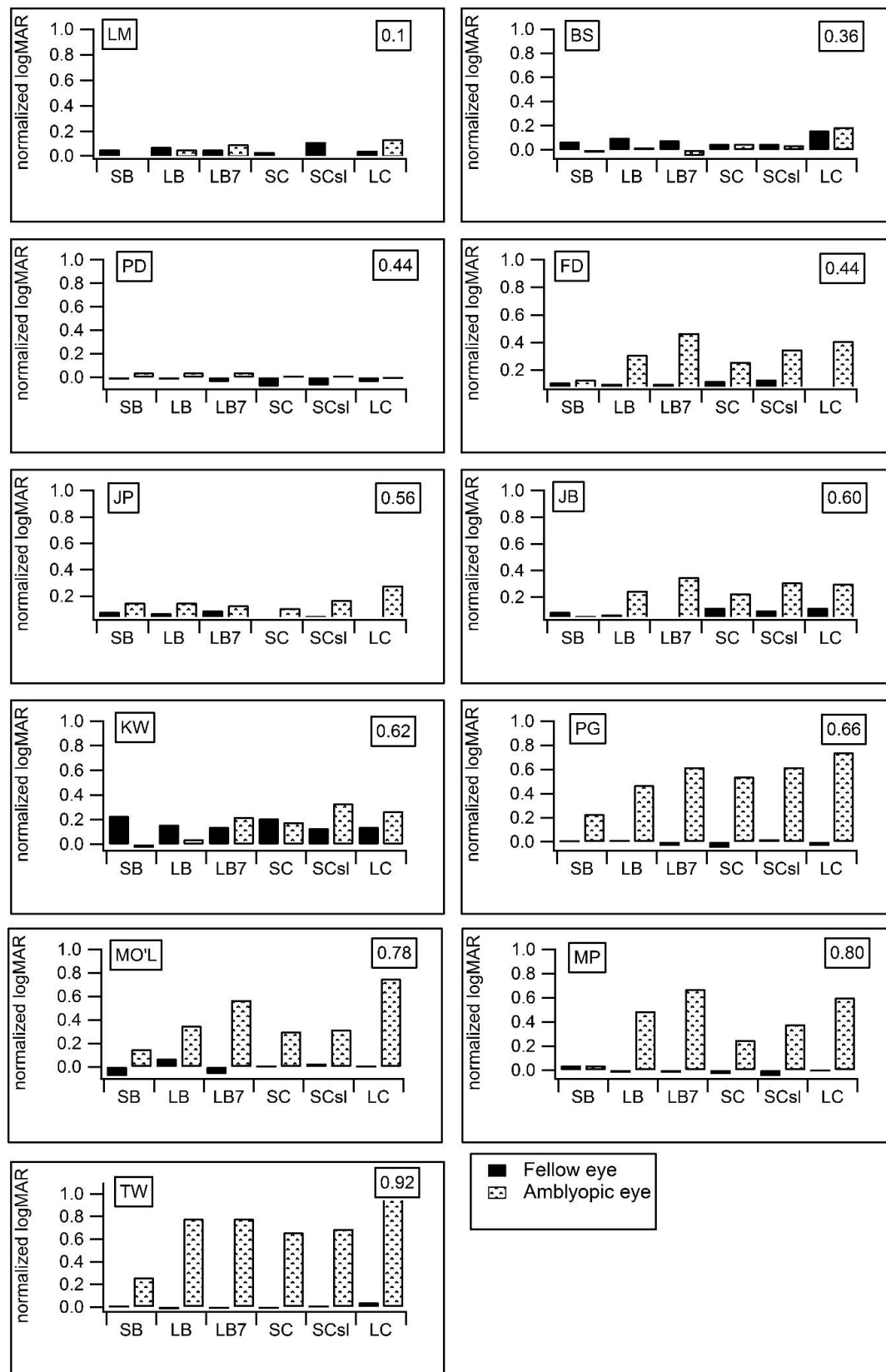


Figure 1. LogMAR for the amblyopic (dotted bars) and fellow (black bars) eyes for each of 11 adults with strabismic and mixed strabismic/anisometropic amblyopia, normalized to the uncrowded logMAR for each of the crowded conditions. LogMAR in the amblyopic eye as derived from initial screening is shown in the top right corner. An example of each display is shown in the key for Figure 2.

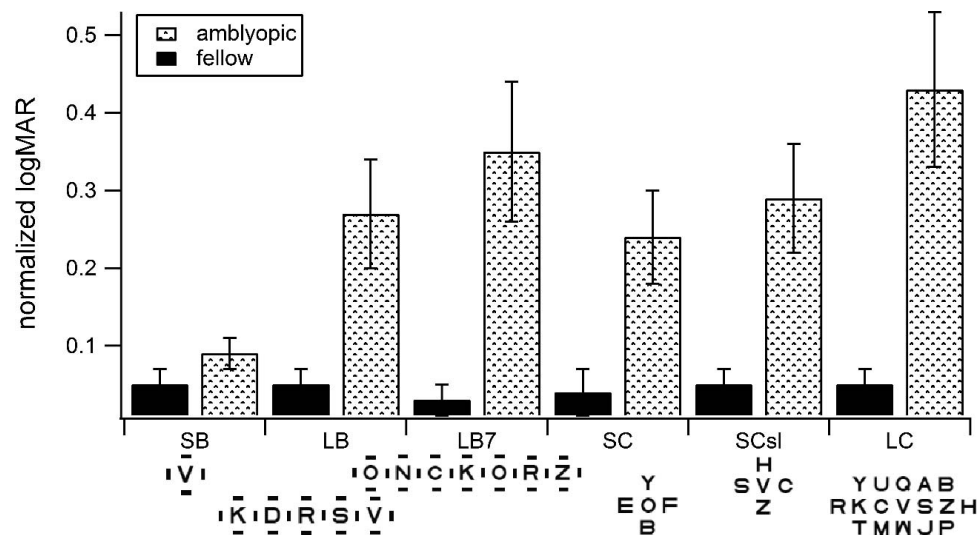


Figure 2. Mean logMAR for the amblyopic (dotted bars) and fellow (black bars) eyes of 11 adults with strabismic and mixed strabismic/anisometropic amblyopia, normalized to the uncrowded logMAR for each of the test conditions. An example of each display is shown in the key. Edge-to-edge target-flanker separation was 0.5 letter width in each test. Error bars represent  $\pm 1$  standard error.

## Effect of single-letter versus linear test presentation

In the amblyopic eyes, there was more crowding in the linear than the single-letter test conditions. Mean, normalized logMAR was significantly poorer in both the line tests with bar flankers (LB, LB7) than in the single-letter test with bar flankers (SB;  $p < 0.01$ ). The seven-letter test (LB7) showed a trend toward more crowding than the five-letter version (LB), but the difference between the two was not significant ( $p = 0.44$ ). Mean, normalized logMAR was also significantly poorer in the line test with letter flankers (LC) than in the single-letter test with letter flankers (SC;  $p < 0.05$ ).

## Error analysis

Figure 3 shows the relative percentages of the different error types in the line tests (LB, LB7, LC) in the amblyopic and fellow eyes of the participants with strabismic and mixed strabismic/anisometropic amblyopia. Light-gray shading shows random errors, dark shading shows adjacent left errors, and diagonally striped shading shows adjacent right errors.

The first analysis examined whether the adjacent errors were anything other than chance in each of the line tests. Because there were two adjacent letters from nine possible Sloan letters other than the target letter, the probability of naming an adjacent letter correctly by chance was 2/9, or 0.22. For the line tests with bar flankers (LB, LB7), on average the frequency of adjacent errors when viewing was with the fellow, nonamblyopic eyes was not significantly different from expectations resulting from chance (LB:  $\chi^2 = 3.44$ ,  $p = 0.06$ ; LB7:  $\chi^2 = 2.08$ ,  $p = 0.15$ ); but for the amblyopic eyes, more adjacent errors occurred than would be expected from chance (LB:  $\chi^2 = 9.69$ ,  $p < 0.05$ ; LB7:  $\chi^2 = 21.31$ ,  $p < 0.001$ ). In the line test with letter flankers (LC), on average more adjacent errors occurred than would be expected from chance for both amblyopic ( $\chi^2 = 37.51$ ,  $p < 0.001$ ) and fellow eyes ( $\chi^2 = 4.88$ ,  $p < 0.05$ ).

The second analysis examined the frequency of right and left adjacent errors in the line tests with bar and letter flankers (LB and LC, respectively). In test LB7, Figure 3 shows a large proportion of adjacent right errors in the amblyopic eyes, although the number of some error types was too low to enable a chi-square analysis. In the line test with bar flankers (LB), the proportion of right and left errors was not different for amblyopic ( $\chi^2 = 0.07$ ,  $p = 0.80$ ) or fellow eyes ( $\chi^2 = 0.33$ ,

| Eyes           | SB          | LB          | LB7         | SC          | SCsl        | LC          |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Fellow eyes    | 0.05 (0.02) | 0.05 (0.02) | 0.03 (0.02) | 0.04 (0.03) | 0.05 (0.02) | 0.05 (0.02) |
| Amblyopic eyes | 0.09 (0.03) | 0.27 (0.07) | 0.35 (0.09) | 0.24 (0.06) | 0.29 (0.07) | 0.43 (0.10) |

Table 3. Mean, normalized logMAR (standard error) for the six test conditions for the fellow and amblyopic eyes of the participants with strabismus.



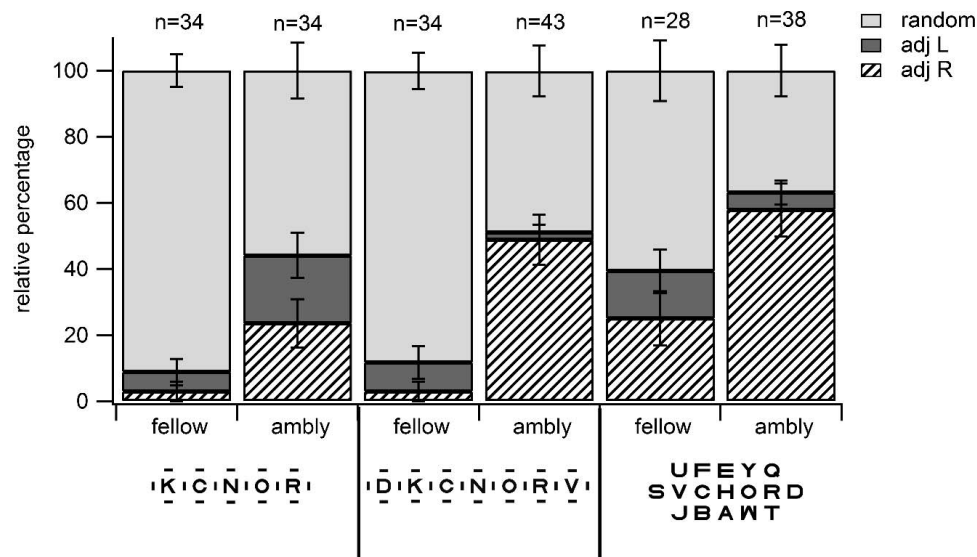


Figure 3. Relative percentages of the different error types in the linear tests (LB, LB7, LC) for the amblyopic and fellow eyes of the participants with strabismic and mixed strabismic/anisometropic amblyopia. Light-gray shading shows random errors, dark shading shows adjacent left errors, and diagonally striped shading shows adjacent right errors. Error bars represent  $\pm 1$  standard error. The total number of errors analyzed for each condition is shown above the bars.

$p = 0.56$ ). However, in the line test with letter flankers (LC), there were more right than left adjacent errors for both amblyopic ( $\chi^2 = 6.24$ ,  $p < 0.05$ ) and fellow eyes ( $\chi^2 = 5.14$ ,  $p < 0.05$ ).

The third analysis examined whether the frequency of adjacent errors using the single-letter test with Sloan letter flankers (SCsl) was anything other than random. Figure 4 depicts the relative proportions of adjacent and random errors occurring with test SCsl. Here, adjacent errors corresponded to one of the surrounding four letters, while any other error was deemed random. The probability of an adjacent error occurring by chance was therefore higher than before at 4/9, or 0.44. On average, there were more adjacent errors than would be expected from chance for both amblyopic ( $\chi^2 = 9.97$ ,  $p < 0.05$ ) and fellow eyes ( $\chi^2 = 4.00$ ,  $p < 0.05$ ).

The fourth analysis looked for a difference in the frequency of adjacent and random errors between amblyopic and fellow eyes in the single-letter test SCsl. There was no difference in the proportion of random and adjacent errors in the amblyopic ( $\chi^2 = 0.19$ ,  $p = 0.67$ ) or fellow eyes ( $\chi^2 = 0.23$ ,  $p = 0.63$ )—that is, amblyopic and nonamblyopic eyes did not differ in relative proportion of adjacent versus random errors.

eyes of a group of adults with strabismic and mixed strabismic/anisometropic amblyopia. In common with other reports, we found marked variability of crowding among participants (Bonne et al., 2004; Polat, Ma-Naim, Belkin, & Sagi, 2004; Regan et al., 1992). On average, there was a greater elevation of crowding seen

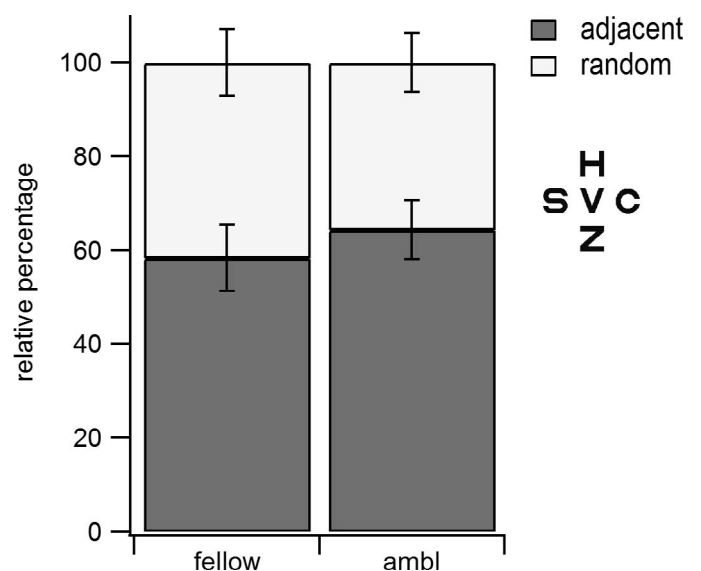


Figure 4. Relative percentages of error types—adjacent (horizontal and vertical) and random—for amblyopic and fellow eyes of the participants with strabismic amblyopia for test SCsl, an example presentation of which is shown on the right. Light-gray shading shows random errors and dark-gray shading shows adjacent errors. Error bars represent  $\pm 1$  standard error. The total number of errors analyzed for each condition is shown above the bars.

## Discussion

A series of custom-designed visual acuity tests was used to infer the relative influences of linear versus single-letter presentation and target-flanker similarity on visual acuity (logMAR) in the amblyopic and fellow



in amblyopic eyes with letter rather than bar flankers and with linear rather than single-letter presentations. There was also an additive effect, with most crowding occurring in the linear presentation with letter flankers.

In a previous study, we used similarly constructed tests to investigate the effect of test-chart configuration on logMAR in children ages 4–6 and 7–9 and in a control group of adults with normal or corrected-to-normal vision (Norgett & Siderov, 2014). The pattern of crowding in the current study (in a sample of strabismic and mixed strabismic/anisometropic amblyopic eyes) shows a similar pattern to the results arising from young children in our previous study (Figure 5). This similarity between performance of strabismic amblyopic eyes and that of the young children lends strength to the view of amblyopia as a poorly matured visual system. Levi & Carkeet (1993) compared a range of visual functions in people with strabismic amblyopia and in young children. They found that some functions which develop early, such as peak contrast sensitivity, were unimpaired in the individuals with strabismic amblyopia, whereas vernier acuity and grating acuity, which develop later, were impaired. Thus the timeframe in which strabismus exerts its influence on the developing visual system can be inferred. The findings of our study suggest that in strabismic amblyopia, the visual system is affected before the maturation of crowding is complete, at around 6–12 years, depending on the study design (Bondarko & Semenov, 2005; Doron et al., 2015; Jeon, Hamid, Maurer, & Lewis, 2010).

Mean logMAR in test SB—single letters surrounded by flanking bars—was significantly higher than unflanked logMAR in the amblyopic (0.09 logMAR) and fellow eyes (0.05 logMAR), showing the effect of contour interaction on visual acuity with simple bar flankers (Figure 2). The mean normalized logMAR using test SB was not significantly different between the amblyopic and fellow eyes. This result shows that on average, the magnitude of contour interaction scaled with acuity in our participants with amblyopia. Nevertheless, as reported by others (Hess et al., 2001), some individuals showed more contour interaction with their amblyopic eye than their fellow eye (e.g., participants PG and MOL in Figure 1), while for others, elevated contour interaction was not seen (e.g., participants JB and MP), and flanked acuity scaled with unflanked acuity (Figure 1; Flom et al., 1963).

Crowding has been shown to be greater in typical peripheral vision when the flanker and target are from the same perceptual group—that is, all letters rather than a target letter with bar flankers (Kooi et al., 1994; Nazir, 1992). In the amblyopic eyes of our participants, poorer mean logMAR was found in the tests with letter flankers compared to those with bar flankers, implying

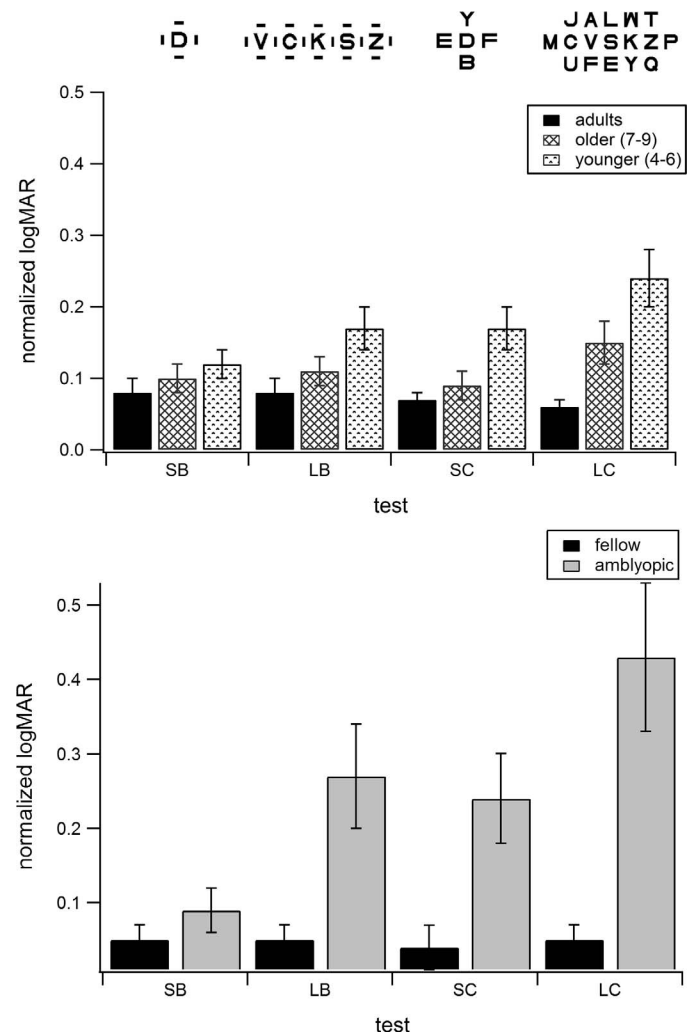


Figure 5. Crowded logMAR of children and adults with normal vision from our previous study (Norgett & Siderov, 2014; top panel) and adults with strabismic and mixed strabismic/anisometropic amblyopia from this study (lower panel) for four crowding conditions for the same test charts. Example presentations of the conditions are shown above the graphs. Top panel: Mean logMAR, normalized to the unflanked acuity, for each of the conditions for younger children (4–6 years; dotted bars), older children (7–9 years; crosshatched bars), and adults (black bars). Bottom panel: Mean logMAR, normalized to the unflanked acuity, for the same four crowding conditions for amblyopic (gray bars) and fellow eyes (black bars) of adults with strabismic and mixed strabismic/anisometropic amblyopia. Error bars represent  $\pm 1$  standard error.

a similar process operating in foveal or extrafoveal viewing in strabismic amblyopia.

Possible mechanisms which may increase crowding in amblyopia include abnormal long-range lateral interactions (Bonneh et al., 2004; Polat et al., 1997), excessive feature integration (Levi et al., 2002; Pelli, Palomares, & Majaj, 2004) and extended pooling (Hariharan et al., 2005). The current findings are

consistent with the theory that target and flankers are integrated within a receptive field, and where letter flankers are used, the target cannot be isolated for selection as easily as when bar flankers are used. The error analyses of the tests with letter flankers (Figures 3 and 4) provide evidence that in amblyopic eyes, a neighboring letter is selected in favor of the target letter at a level greater than would be expected by chance. The specific interaction of the flanker identity with the target could be the reason for more crowding with letter than bar flankers (Dakin et al., 2010). When bar rather than letter flankers are used, the bar may be less likely to change the percept of the target letter because the bars do not provide an anchor or similar feature to confuse with the target letter (Bernard & Chung, 2011), nor do they form part of a meaningful group with the target (Reuther & Chakravarthi, 2014). There is also evidence that the number of flanker features within a receptive field contributes to crowding, particularly if they are sufficiently similar to the target to be grouped with it perceptually (Bernard & Chung, 2011; Manassi, Sayim, & Herzog, 2012; Saarela, Sayim, Westheimer, & Herzog, 2009). This idea could help explain the greater crowding found in our single-letter tests with letter rather than bar flankers. The former test contains more features overall than the equivalent test with bar flankers.

A further explanation for the increased crowding in the amblyopic eyes could stem from the findings of Lev et al. (2014), where crowding was shown to be greater where larger perceptive fields are used. In foveal viewing in amblyopic eyes, similar to with young children, larger processing units may be used (Doron et al., 2015).

Most participants showed poorer mean logMAR in their amblyopic eyes in the linear tests than in the corresponding single-letter tests (Figure 1), and the same was true for the group means (Figure 2). This finding is not surprising, given the extra oculomotor demand required to complete the linear tests and the fact that both oculomotor deficits and positional uncertainty have been shown to be present in strabismic amblyopia (Chung, Kumar, Li, & Levi, 2015; Ciuffreda, Kenyon, & Stark, 1980; Hess & Holliday, 1992; Levi et al., 1987). In a study which tracked eye movements in nonamblyopic and amblyopic eyes during a reading task, Kanonidou, Gottlob, and Proudlock (2014) showed that individuals with strabismic amblyopia made more saccades per line when reading small print with their amblyopic eye, showing poorer control of eye movements during reading.

The results of the error analysis of linear tests showed that more adjacent errors (i.e., left or right errors) occurred in the amblyopic eyes of participants in all of the linear tests than would be expected by chance only (Figure 3). This result supports the view

that increased positional uncertainty or oculomotor demands in amblyopic vision led to poorer performance in these tests. In addition, in the tests where seven letters were read (LB7, LC), more rightward than leftward errors were made, which suggests that as observers completed the longer letter-string tests, they were missing a letter or letters. Such results are consistent with findings in observers with typical vision, in which more errors were made when participants read longer rather than shorter letter strings (Bedell et al., 2015).

It was not possible with the experimental conditions used in this study to distinguish whether positional uncertainty or oculomotor errors caused the participants to lose their place more frequently when reading the line of letters with their amblyopic eyes. Because positional uncertainty can lead to a degraded sensory signal, causing increased saccadic drift (Chung & Legge, 2009; González et al., 2012), it may be that a combination of the two factors is present.

Positional uncertainty could also cause more crowding in the amblyopic eyes by increasing the likelihood of misbinding of features from flanking elements with the target, leading to excessive feature integration (Bernard & Chung, 2011; Chung & Legge, 2009). The error analysis of test SCsl (Figure 4) showed that near threshold, participants were naming the flanking letters at a frequency greater than predicted by chance—but crucially, the proportion of adjacent errors was not greater in amblyopic eyes than fellow eyes. If it is assumed that the fellow eyes of people with strabismic amblyopia are not prone to positional uncertainty or fixational errors any more than eyes with typical vision, then it follows that a factor other than positional uncertainty or poor fixation in the amblyopic eyes is responsible for the greater crowding measured in the single-letter test with letter flankers than in the equivalent test with bar flankers. This finding is consistent with that of Bonnef et al. (2007), who used briefly presented stimuli and concluded that unsteady fixation could not be the only cause of increased crowding in amblyopia.

Participants in our study were given unlimited time to view the charts, and our observation was that most took longer to complete the testing with their amblyopic than fellow eyes. Previous studies have shown a temporal element to crowding in typical vision (Lev & Polat, 2015; Lev, Yehezkel, & Polat, 2014; Tkacz-Domb & Yeshurun, 2017) and in strabismic amblyopia (Bonnef et al., 2007). It takes longer to isolate targets from flanking elements in strabismic amblyopia, possibly because of a slower or more sustained inhibition. Had we limited the time given to read the charts, this would most likely have produced an even greater difference in crowding between amblyopic and nonamblyopic eyes.

## Clinical implications

Our results lend support to the recommendation that letter flankers, not bars, be used in visual-acuity screening tests (Lalor, Formankiewicz, & Waugh, 2016; Song, Levi, & Pelli, 2014). In addition, use of a linear test could help identify children with poor gaze control. Persistent amblyopia has been linked to poor gaze control (Birch, 2013), so treatment for children showing poor performance on a linear test could focus on strategies which minimize the disruption to binocular input (Subramanian, Jost, & Birch, 2013).

## Conclusions

Our results show that similar to young children, crowding in adults with strabismic and mixed strabismic/anisometropic amblyopic eyes is dependent on stimulus and task demands. The more precise eye-movement control required to read a string of letters or positional uncertainty and the increased target-flanker similarity of letter flankers increase crowding in this group. These two factors can have an additive effect, with the poorest performance being in the test with linear presentation with letter flankers.

*Keywords:* amblyopia, visual crowding, visual acuity

## Acknowledgments

The authors are grateful for the suggestions of two anonymous reviewers.

The Sloan font was downloaded from <http://psych.nyu.edu/pelli/software.html> (Pelli et al., 1988).

Commercial relationships: none.

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